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LYMPHOCYTIC CHORIOMENINGITIS

by

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ABSTRACT

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Lymphocytic choriomeningitis (LCM) is an enzootic, viral disease of lower animals, which when present, is a hazard to people who work with laboratory animals for the following reasons. Being prevalent in wild rodents, it readily spreads to most species of laboratory animals unless specific precautions are taken to keep it out. Although generally occurring in a subclinical form it is so readily transmitted directly, indirectly, or via insect vectors that it may become widely spread throughout an animal colony before its presence is known. From experimental animals it has frequently contaminated other viruses being studied, invalidating results. Contact with infected animals and contaminated materials has resulted in numerous human cases varying in severity from inapparent to rarely fatal. As the name implies, in its acute form it produces an encephalomyelitis with lymphocytic infiltration of the choroid plexus, meninges and ependyma. The immunologic character of the disease in mice varies with the manner of infection. Periodic checking of an animal colony for the presence of LCM is recommended.

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LYMPHOCYTIC CHORIOMENINGITIS[1]

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Lymphocytic choriomeningitis is an enzootic, viral disease of lower animals, which is transmissible to man. Although usually subclinical, in its acute form, it produces an encephalomyelitis with lymphocytic infiltration of the choroid plexus, meninges and ependyma. Specialization in research requires many workers to use experimental animals who have no direct interest in the diseases of laboratory animals. Consequently, in many instances people learn about individual diseases of laboratory animals only when they interfere with their research. In the case of lymphocytic choriomeningitis this approach may be both costly and dangerous for the following reasons:

1. Lymphocytic choriomeningitis is so prevalent in wild rodents and so likely to be encountered in commercially available laboratory animals that special precautions are necessary to keep it out of the colony.

2. All of the ordinary laboratory animals are susceptible.

3. Most cases are subclinical so that the disease may become widely spread throughout a colony before associated research workers and attendants are aware of it. Although present in an inapparent form it is readily transmitted.

4. Being present in a silent form, it may contaminate other viruses used experimentally in the laboratory, thereby invalidating results and causing embarrassment. The virus was first isolated in 1934 by Armstrong and Lillie while studying tissues from a patient presumed to have died of St. Louis encephalitis. On several occasions, tissues or sera from infected laboratory animals have introduced lymphocytic choriomeningitis virus as a contaminant of other viruses being passed in the laboratory.

5. Man is susceptible to LCM and people working with infected laboratory animals may be heavily exposed. In its rare but most acute form, it produces a fatal meningoencephalitis in man.

Distribution

LCM is widespread throughout the United States, Europe, Asia, Africa, and probably the world. Surveys of wild house mice usually reveal about 10% to be infected. Armstrong (1940) found 21.5% to be infected in Washington, D. C. The virus has been encountered in dogs which also had distemper (Dalldorf, 1939).

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[1] The animals used in this study were handled in accordance with the "Principles of Laboratory Animal Care" established by the National Society for Medical Research.

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Etiology

Strains of LCM virus vary in their pathogenicity but there is no evidence of different antigenic types (Olitsky & Casals, 1948). The virus is filterable through the usual bacteria retaining filters and is from 33 to 60 mμ in size (Van Rooyen & Rhodes, 1948). It is rapidly inactivated by ultraviolet light and killed by 55° C in 20 minutes. It has been cultivated in tissue cultures and chick embryos. The virus retains its viability when frozen or when dried and stored below 0°C. Virus is present in [illegible] and fluids of infected animals.

LCM has been found to occur naturally in mice, guinea pigs, chinchilla, cotton rats, foxes, dogs, monkeys, and man. Species which have been infected experimentally include rabbits, hamsters, squirrels, and horses. Infections are usually subclinical in the hamster, ferret, rabbit, dog, and horse. Cattle, pigs, cats, and chickens do not appear to be susceptible.

Transmission occurs readily by direct contact via the conjunctiva, respiratory or digestive tracts, or through the intact skin. The ease with which the virus may be transmitted to man has been amply illustrated by the many cases which have occurred in homes and laboratories. Numerous human cases have been reported in homes where infected mice were subsequently found (Armstrong & Sweet, 1939). In most such cases, contaminated food or dust appears to have been the most likely source of infection. In several instances human cases have occurred from direct contact with infected mice. A common source of infection for laboratory workers has been the urine of infected mice. In some such instances the worker did not know of any breaks in the skin of his hands. Three fatal cases occurred in people working with canine distemper virus vaccine (Armstrong, 1942). Man may be infected by blood-sucking insects (Armstrong, 1955). The virus has been isolated from ticks which carry the virus through their life cycle. Nymphs have transmitted the disease to guinea pigs (Shaughnessy & Milzer, 1939). *Aedes aegypti* mosquitoes have transmitted the virus experimentally to guinea pigs, and monkey lice have transmitted the virus to other monkeys on two recorded occasions (Armstrong, 1942; Milzer, 1942). Laboratory workers have been infected by getting the virus into their eyes (Hays and Hartman, 1943).

Human infections with LCM may vary in severity from being asymptomatic to fatal. The frequency of asymptomatic infections, as well as the incidence of the disease, may be inferred from a survey conducted by Armstrong (1955) in which he found 12% of 2000 human sera collected at random to show neutralizing antibodies. The people with neutralizing antibodies had no history of a clinical attack. Three forms of clinical infection with the disease include: (1) An influenza-like illness with no other involvement. (2) The influenza-type infection may be followed by meningeal symptoms. (3) There may be a meningoencephalitis, sometimes fatal. When fatal, it usually produces a severe, non-suppurative encephalomyelitis with lymphocytic infiltrations of the meninges, choroid plexus,

and ependyma. There may also be perivascular infiltrations, engorgement, edema, focal gliosis, and necrosis.

There are three forms of infection in mice, depending upon the manner of infection whether congenital, natural, or experimental. Mice infected in utero or when inoculated during the first 7 or 8 days after birth develop an infection which has several peculiar features. Most such mice appear normal yet harbor the virus as long as they live. They develop no circulating antibodies but are resistant to a clinical infection with lymphocytic choriomeningitis virus. The tissues, fluids, and exudates of such mice are highly infectious, and the virus readily spreads from them to other mice in the colony. The virus is passed to their offspring and such congenital passage has been demonstrated for at least 15 generations by workers at the National Institutes of Health. According to Traub (1941) the presence of latent lymphocytic choriomeningitis virus favors the development of lymphomatosis in mice.

Natural infections which occur in mice after weaning differ from those which are congenitally infected in several respects. Naturally infected mice develop both complement fixing (CF) and neutralizing antibodies. Their offsprings are susceptible. About 20% of them will show signs of clinical illness. Clinical illness usually occurs in mice under 6 weeks of age. Less than 2% of the clinically ill mice die. With infected mice in a colony, usually over 50% of the individuals in the colony will become infected. Clinical signs of infection, though non-pathonomonic, are suggestive. Such mice appear rough, drowsy and tend to sit in a corner by themselves. They become emaciated, develop photophobia, and conjunctivitis. They become weak but there is no paralysis. They tend to move only when pushed and their movement is slow, stiff, and creeping. Because of the emaciation their legs often appear too long. Any such suspicious animals should be removed from the colony, and it is from such mice that virus can most often be isolated. There are numerous factors which influence LCM immunity in the mouse, making this an interesting immune mechanism for study (Rowe, 1954).

A method for the recovery of virus from suspect mice is to remove the brain, spleen, and heart blood from a group of 4 suspects. These tissues may be weighed, pooled and ground in a 20% concentration in buffered saline at pH 7.6. Centrifugation at 3000 RPM for 10 minutes will provide a sufficiently clear supernate for inoculation. The supernate should be cultured for bacterial contamination and subsequently, 3 to 6-week-old mice may be inoculated intracerebrally with .03 ml. and intraperitoneally with .25 ml. Because some strains of LCM virus are more readily isolated in guinea pigs than in mice, it is also well to inoculate young guinea pigs intraperitoneally with .5 ml. of the suspect tissue supernate. The remaining inoculum can be frozen and if later shown to be bacteriologically contaminated, penicillin and streptomycin may be added followed by re-inoculation of more animals. Inoculated mice should be held for 21 days. If lymphocytic choriomeningitis is present, symptoms will usually appear between the 5th and 7th day after inoculation.

Mice under 6 weeks of age, experimentally infected intracerebally, become weak, tend to sit alone, have roughened fur, and frequently develop convulsions. These convulsions may be elicited in infected mice by twirling them by the tail. During a convulsive seizure the hind legs are characteristically stretched out stiffly, the tail rigid, the back humped, and the front legs move rapidly. They may drag themselves around for a few minutes by their front feet. They usually die in such a convulsive seizure with their hind legs extended in rigor. Death usually occurs in from 1 to 2 days after the onset of convulsions.

Gross lesions which develop in mice, whether infected naturally or experimentally, are meager. There may be a pleural exudate, a fatty liver, and enlarged spleen. Microscopically, there is usually a round cell infiltration, predominantly lymphocytic, which is most striking in the meninges especially at the base of the brain, in the choroid plexus, and in perivascular lymph spaces of submeningeal vessels. In general, the lymphocytic infiltrations in the brains of naturally infected cases are not so severe as in the experimentally ones which have been inoculated intracerebrally. The lungs frequently show peribronchiolar and perivascular infiltrations with round cells and a slight thickening of alveolar walls. Small collections of lymphoid cells are frequentiy present in the liver near blood vessels and scattered through the parenchyma. There may be necrosis of hepatic cells in areas of lymphocytic infiltration. A patchy reticulo-endothelial hyperplasia occurs in the spleen.

Naturally infected mice which have only a subclinical infection may be made to reveal this infection by the intracerebral injection of a non-infectious foreign material such as broth, serum, or a normal tissue suspension. The inoculation of foreign material into the brain tends to cause the virus to localize there, resulting in the same syndrome as if these mice had been inoculated intracerebrally with the virus. Such attempts to induce clinical infection should be done in mice under 6 weeks of age.

Although hamsters may be infected experimentally, the infections are not only subclinical but they fail to produce either significant gross or microscopic lesions although the virus may be present in very high titer. In the guinea pig, a viral pneumonia and pulmonary edema are likely to occur in addition to gross lesions present in the mouse. Histologic lesions in the brain of the guinea pig are similar to those found in the mouse.

The infection of laboratory animals with LCM is obviously very undesirable. Its presence is likely to invalidate the results of experiments and it is a hazard to human health. To develop and maintain a mouse colony free of LCM requires that disease-free breeding stock be obtained and kept isolated from wild rodents. The use of disease-free pelleted feeds and of bedding, such as shavings, from a source not exposed to wild rodents is essential. Animal quarters must be free of insects and external parasites which are potentially capable of transmitting the infection. Attendants must be alert to the fact that most species of laboratory animals are susceptible, that dogs and monkeys have been the source of infection

in laboratories and that mouse colonies should be kept separate from other species. Checks for latent virus should be made periodically, particularly in mice. If numerous intracerebral inoculations of control mice are made in the course of routine work then latent natural infections would be detected. Laboratory workers must be alert to the fact that the virus is capable of penetrating the intact skin and that the urine of infected laboratory animals may contain the virus.

REFERENCES

ARMSTRONG, C., AND LILLIE, R. D. Experimental lymphocytic choriomeningitis of monkeys and mice produced by a virus encountered in studies of the 1933 St Louis encephalitis epidemic. *Pub. Health Rep.*, **49**: 1019, 1934.

ARMSTRONG, C., WALLACE, J. J., AND ROSS, L. Lymphocytic choriomeningitis: gray mice, *mus musculus*, a reservoir for the infection *Pub. Health Rep.*, **55**: 1222, 1940.

ARMSTRONG, C., AND SWEET, L. K. Lymphocytic choriomeningitis; report of 2 cases with recovery of virus from gray mice, *Mus musculus*, trapped in two infected households. Pub. Health Rep. **54**: 673–684, 1939.

ARMSTRONG, C. Some recent research in the field of neurotropic viruses with especial reference to lymphocytic choriomeningitis and herpes simplex. *Mil. Surg.*, **91**: Aug. 1942. (Kober Lecture.)

ARMSTRONG, C. in T. G. HULL, Diseases Transmitted from Animals to Man. 4th ed, 1955, Charles Thomas & Co., p. 633.

DALLDORF, G. The simultaneous occurrence of the viruses of canine distemper and lymphocytic choriomeningitis. J. Exper. Med. **70**: 19–27, 1939.

HAYS, G. S., AND HARTMAN, T. L. Lymphocytic choriomeningitis (report of a laboratory infection). *Bull. Johns Hopkins Hosp.*, **73**: 275, 1943.

MILZER, A. Studies on the transmission of lymphocytic choriomeningitis. *J. Infect. Dis.*, **70**: 152, 1942.

OLITSKY, P. K., AND CASALS, J. Viral encephalitides. In Viral and Rickettsial Infections of Man (Rivers, T. M., ed.). Philadelphia, J. B. Lippincott Co., 1948, p. 194.

ROWE, W. P. Studies on Pathogenesis and Immunity in Lymphocytic Choriomeningitis Infection of the Mouse. Research Report, Naval Medical Research Institute, National Naval Medical Center, Bethesda, Md., 27 May 1954, v. 12.

SHAUGHNESSY, H. J., AND MILZER, A. Experimental infection of *Dermacentor andersoni* stiles, with the virus of lymphocytic choriomeningitis. *Ann. J. Pub. Health*, **29**: 1103, 1939.

TRAUB, ERICH. The influence of latent lymphocytic choriomeningitis infection on the appearance of lymphomatosis in white mice. Zentralbl. Bakt., **147**: 16–25, 1941.

VAN ROOYEN, C. E., AND RHODES, A. J. Virus Diseases of Man. New York, Thomas Nelson & Sons, 1948, p. 1051.